Effects of Nutrifen® and Nutrifen Plus® on reproductive performance and blood profiles of sows

By

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Declaration:

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Summary

In the livestock industry, plant-derived products are used as feed additives in order to improve production. Research on phytogenic feed additives has increased in recent years because of the ban on most antibiotic feed additives within the European Union in 1999, which was due to concerns about the development of antibiotic-resistant pathogenic bacteria. There is a vast variety of phytogenic feed additives available, which include spices, herbs and essential oils. Fenugreek (*Trigonella foenum-graceum*) is a member of the leguminosae family. This annual plant is both a medicinal and culinary herb which has been used for centuries and is mainly cultivated in Northern Africa, Southern Asia and India. Both the leaves and seeds of this herb have been utilised extensively to prepare powders and extracts for medicinal purposes. The seeds have antibacterial and galactogogue properties and stimulate the digestive system in humans . Literature on the use of fenugreek in pigs is limited; with most research having been done on humans. This study was therefore conducted to evaluate the effects of fenugreek supplementation on sows and their litters on their reproductive and production performance. The trial utilised 120 sows and their 1480 piglets and extended from the 85th day of gestation until the piglets were weaned at an age of 28 days. The sows were housed in individual crates in the dry sow house and in farrowing crates during lactation. Two commercial fenugreek products, Nutrifen® and Nutrifen Plus®, were used at the levels recommended by the manufacturer. The different treatments were: 1) control (CON), with no fenugreek supplementation; 2) sows supplemented with 0.2% Nutrifen®; 3) sows supplemented with 0.2% Nutrifen Plus®. The main objective of this study was to determine the effects of fenugreek supplementation during the last trimester of gestation and during lactation on sow reproductive performance and litter parameters. The production parameters measured were the number of piglets born alive, the number of stillborn piglets, the number of mummified piglets, the litter birth weight (kg), the pre-weaning mortality (%), the piglets weaned per sow, the litter weaning weight (kg), the back fat thickness (mm) of the sows at weaning and the total feed intake during lactation (kg). The secondary objective was to evaluate the effect of fenugreek supplementation during the last trimester of gestation and during lactation on the immunity of sows and their piglets. The biomarkers measured were the white blood cell count, red blood cell count, lymphocyte and immunoglobulin G levels. There was a significant effect ($P = 0.025$) of the fenugreek supplementation on the back fat thickness of the sows at farrowing but not on the back fat loss during lactation, which is an important factor for subsequent reproductive performance. Overall, there was no significant effect of the fenugreek treatments on the sow reproductive performance, the litter parameters or the blood profiles. Further research is needed to establish the full potential of fenugreek in pigs because the mode of action of fenugreek is still not clear.

Opsomming

In die veebedryf word plantekstrakte al hoe meer algemeen gebruik om produksie van diere te verbeter. Navorsing het die afgelope jare baie toegeneem rakende plant ekstakte wat dien as natuurlike groeipromotors. Dit is as gevolg van die verbanning van die meeste antibiotika gebasseerde groeipromotors in die Europese Unie in 1999 weens die feit dat patogeniese bakterië weerstanbiedig teen antibiotika raak. Daar is ‘n groot verskeidenheid plant gebasseerde groeipromoters beskikbaar wat kruie, speserye en essensiële olies insluit. Fenugreek (Trigonella foenum-graceum) is ‘n meerjarige plant en vorm deel van die peulplant familie. Dit word al vir eeue gebruik vir medisinale doeleindes en as krui in die kookbedryf. Die plant word hoofsaaklik verbou in Noord-Amerika, die suidelike gedeelte van Asië en Indië. Beide die blare en die sade van die kruid word grootskaals gebruik vir die vervaardiging van poeiers en ekstrakte vir medisinale doeleindes. Die sade het ‘n antibakteriese effek en kan ook laktasie en die spysverteringsproses bevorder. Literatuur rakende die effek van fenugreek in varke is beperk en die meeste resultate is oor mense gevind. Daarom is daar besluit om ‘n proef uit te voer om die effek van fenugreek op sôe en hul werpsels te evalueer. Die proef het bestaan uit 120 sôe en hul 1480 varkies. Die proeftydperk was vanaf 85 dae dragtigheid tot-en-met die varkies gespeen was op 28 dae. Die behuising van die sôe gedurende die dragtigheidsperiode was in individuele kratte en gedurende laktasie in individuele kraamkratte. Twee kommersiële fenugreek produkte, Nurifen® en Nurtrifen Plus®, was gebruik teen insluitingsvlakke deur die verskaffer aanbeveel. Die verskillende behandelings was: 1) kontrole groep (CON) met geen byvoeging; 2) Nurifen ® byvoeging teen 0.2% insluiting; 3) Nutrifen Plus® byvoeging teen 0.2% insluiting. Die hoofdoel van hierdie eksperiment was om die effek van fenugreek-byvoeging vanaf 85 dae dragtigheid en gedurende laktasie op die sog en werpsel produksie parameters te toets. Die produksie parameters wat vir die varkprodusent belangrik is, sluit in aantal varkies lewendig gebore, aantal varkies dood gebore, aantal gemummifiseerde varkies, die werpsel geboortegewig (kg), die voorspeense mortaliteit van die varkies (%), aantal varkies gespeen per sog, die werpsel speengewig (kg), die spekdikte (mm) van die sôe op speen en die totale voerinname (kg) gedurende laktasie. Die tweede doel was om die effek van ‘n fenugreek aanvulling op immuniteit van sôe en hul werpsels te evalueer. Die proeftydperk was dieselfdeDie biomerkers wat gebruik was, was witbloedseltelling, rooibloedseltelling, limfosiete en immunoglobuliene G. Vanuit die studie is getoon dat die fenugreek-aanvulling ‘n effek gehad het op die spekdikte van die sôe op speen ($P = 0.025$), maar daar was egter nie ‘n effek op die spekdikte verlies gedurende die laktasie nie. Die spekdikte verlies is die belangrike faktor vir toekomstige produksie. Geen groot verskille is waargeneen met die fenugreek-aanvullings op die sog en werpsels se produksie parameters asook hul bloedprofiele nie. Verdere navorsing is nodig om die volle potensiaal van fenugreek in varke te ondersoek.

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Notes

The language and style used in this thesis are in accordance with the requirements of the South African Journal of Animal Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

Abbreviations

DE	Digestible energy
DF	Dietary fibre
ME	Metabolisable energy
HP	Heat production
IgG	Immunoglobulin G
IGF	Insulin-like growth factors
LYM	Lymphocyte
NSP	Non-starch polysaccharides
PIC	Pig improvement company
RBCC	Red blood cell count
WBCC	White blood cell count

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Chapter 1

Introduction

In South Africa, the pig industry is relatively small, only accounting for 7% of the country's total meat consumption in 2013. However, pork consumption has increased by 53% over the last 10 years and continued expansion is projected for the coming decade. Currently South Africa is a net importer of pork, and meat imports have substantially increased over the past decade, with the majority of this being pork and poultry. In 2012, pork imports accounted for 15% of the domestic market, indicating that they play an important role in the South African market. Globally, South Africa only contributes 0.18% of the market, making it an insignificant role player and very vulnerable to changes in the international pork market (BFAP, 2014).

In the livestock industry, plant-derived products are used as feed additives in order to improve production. Research on phytogenic feed additives has increased in recent years because of the ban on most antibiotic feed additives within the European Union in 1999, which was due to concerns about the development of antibiotic-resistant pathogenic bacteria. There are a vast variety of phytogenic feed additives available, which include spices, herbs, essential oils and oleoresins (substances prepared using solvent extraction processes) (Windisch *et al*., 2008).

Fenugreek (*Trigonella foenum-graceum*) is a member of the leguminosae family (Hamden *et al*., 2010). This annual plant is both a medicinal and culinary herb which has been used for centuries and is mainly cultivated in Northern Africa, Southern Asia and India (Sauvaire *et al*., 1991; Shim *et al.,* 2008). The medicinal uses of fenugreek for humans vary from wound healing, reducing blood sugar and cholesterol and promoting lactation (Acharya *et al*., 2006). Both the leaves and seeds of this herb have been utilised extensively to prepare powders and extracts for medicinal purposes.

Fenugreek seeds have antibacterial and galactogogue properties and stimulate the digestive system (Srinivasan, 2006). Chemical analysis of the seeds indicates that they are a rich source of protein, mucilage, non-starch polysaccharides and saponins (Rao & Sharma, 1987). Saponins are known to improve immune function (Ilsley *et al*., 2005), and are converted in the gastrointestinal tract to sapogenins, which may be responsible for lowering cholesterol levels (Smith, 2003).

The use of fenugreek as a galactogogue in humans is reported as far back as 1945, with women showing an increase in milk production 24 – 72 hours after the consumption of fenugreek (Gabay, 2002). Dioscin, a component of fenugreek, is a steroid saponin with a structure similar to that of oestrogen (Muraki *et al.,* 2011). It stimulates the production of growth hormone by binding to the pituitary cells (Hwang *et al.,* 2014). Growth hormone, in turn, has a galactopoietic effect, which could provide an explanation for the mechanism of action of fenugreek on lactation (Alamer & Basiouni, 2005). However, the effect of fenugreek on milk yield is still unclear and further research is needed to clarify its mechanism of action (Al-Shaikh *et al.,* 1999).

Milk production of the sow is considered the first limiting factor for the pre-weaning growth of the piglets. This is the only source of energy for the young piglet until they receive creep feed. Therefore, for optimal growth the sow must have high milk production (Farmer *et al*., 2000). In the new-born piglet the primary reason for

mortality is an inadequate intake of colostrum, with suboptimal colostrum intake also potentially leading to infections. This makes the piglet more susceptible in the postnatal period and after weaning (Drew & Owen, 1988). The new-born piglet is the most susceptible to pathogens relative to the other production stages. Immunologically the piglet is underdeveloped because of a lack of exposure to antigens and this is exacerbated by their physiological immaturity (Rooke & Bland, 2002).

Study aims

The main objective of this study was to determine the effect of fenugreek supplementation during the last trimester of gestation and during lactation on sow reproductive performance and various litter parameters. The production parameters measured were those of importance for the pork producer and included the number of piglets born alive, the number of stillborn piglets, the number of mummified piglets, the litter birth weight (kg), the pre-weaning mortality (%), the piglets weaned per sow, the litter weaning weight (kg), the back fat thickness (mm) of the sows at weaning and the total feed intake during lactation (kg).

The secondary objective was to evaluate the effects of fenugreek supplementation on the immunity of sows and their piglets. The biomarkers measured were white blood cell count, red blood cell count, lymphocytes and immunoglobulin G levels.

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Literature Review

1.1 Pig production in South Africa

The South African pork industry consists of around 125 000 sows which are owned by 4 000 commercial (ca. 100 000 sows), 19 stud and about 110 smallholder (ca. 25 000 sows) farmers, and there are 46 registered pig abattoirs. Pigs are produced throughout all the provinces of South Africa, with North West and Limpopo being the top producers, contributing 44% of the total production (South African Yearbook, 2013/14).

Pig farming in South Africa can be classified into three sectors. The first and largest sector is the commercial farmers. The majority of these farmers are concentrated in a 200 km radius around Pretoria. These farms maintain a closed herd, strict biosecurity policies, feed commercialized rations and slaughter the pigs at commercial abattoirs. The most common system is the farrow-to-finish system where farrowing, weaning and finishing operations are done by the same farmer. One of the benefits of this system is that the piglets enter the growing stage at cost price, rather than market price, decreasing production costs. The livestock facilities on commercial farms are typically temperature controlled buildings that provide optimal growing conditions (BFAP, 2014). Seventy percent of commercial producers mix their own feed rations on-farm, the rest buy in from commercial feed companies. In the Western Cape, many producers buy in premixed feed rations because they are situated far from the sources of raw materials (Mokoele *et al*., 2015).

A small part of the commercial sector is the commercial free-range farmers, which follow strong biosecurity measures and feed balanced rations, which are premixed or home mixes (Bee *et al.,* 2004). The pigs are either housed outside on pastures and in dirt pens or alternatively indoor housing with outside access is used (Gentry *et al.,* 2002). This is due to the increasing demand by consumers for meat that was not produced in intensive systems (Bee *et al.,* 2004). Free-range pigs have a lower feed intake and slower growth rates but have similar meat quality characteristics (Hoffman *et al.,* 2003). These farmers therefore have a different market because their cost of production is higher than that of the intensive producers.

The second sector consists of small and semi-commercial units. These units have low biosecurity policies, buy from auctions, and frequently move pigs between farms. The feed rations tend to vary from commercial diets to cooked diets and illegal swill feeding. They market their pigs to local markets and only a few to commercial abattoirs (Mokoele *et al*., 2015).

The last sector consists of the informal free-range farmers. The pigs roam free and feed off discarded household scraps. These pigs are slaughtered informally for household consumption or special events (Mokoele *et al.,* 2015).

Just over 2.7 million pigs are slaughtered annually at 153 registered pig abattoirs, with these utilising modern technology and techniques (SAPPO, 2011). The carcasses are allocated to different markets according to weight and the average slaughter weight in South Africa is 78 kg. The pork meat industry has two distinct markets, one for fresh meat and the other for processed products, with the usage by these two markets being approximately equal (Pieterse, 2006). The baconer class, to which carcasses of 66–85 kg are assigned, comprises 70% of the market and is sold mainly to the processing market (Grimbeek *et al.,* 2014).

1.2 Pig breeding in South Africa

The South African stud industry is mainly driven by two companies that together control 80–85% of the market. The Pig Improvement Company (PIC) controls the majority of the market, with 45%, and Topigs South Africa contributes 30%, with Alliance Genetics South Africa making up most of the remaining 5–10%. The rest of the industry consists of the stud breeders (Visser, 2014). Biosecurity is becoming very important to producers as it is necessary for export; therefore most farmers are implementing semen-only programs to improve their biosecurity standards. This ensures no animal movements into the farm from the outside.

The aforementioned genetic companies develop and sell synthetic breeds referred to as hybrid or crossbred pigs. Advanced testing programs are used to test carcass and production performance and thereby guide selection. One example of a hybrid breed is the Camborough® sow from PIC, which is bred for, amongst other traits, robustness and for producing fast-growing and lean piglets (Kelly *et al.,* 2001). Hybrid sire lines normally contain genetic material from more than two breeds in various percentages.

The predominant pure pig breeds used for commercial farming are the Large White, Landrace and Duroc (Swart *et al.,* 2010). There is a preference for these breeds because of their rapid growth relative to the indigenous breeds (Chimonyo & Dzama, 2007). In South Africa there are two indigenous breeds, namely the Kolbroek and the Windsnyer. These breeds are considered as less efficient than the modern breeds because of their tendency to put on excess fat (Ramsay *et al.,* 2000). The indigenous breeds are used mostly by people in rural small-scale farming systems (Madzimure *et al.,* 2012).

1.3 Nutrient requirements

With genetic progress the modern pork industry demands for improvements in productivity with an emphasis on the nutrient requirements of the animals (Cooper *et al.,* 2001). Management has an important role in determining which feed will optimise production during each stage and allow the realisation of the animal's biological potential. The nutritional requirements of pigs differ between various pig breeds and production stages.

Sows are of central importance in pork production systems. They are the reproductive units of the herd and their genetic potential and productivity determines the maximum production proficiency of the whole system. Although sows numerically represent a small fraction of the herd, their feed usage contributes 20% of the total feed for a farrow-to-finish production unit. A sub-optimal diet can have numerous negative effects on the sow's productivity, including smaller litter sizes and weights, decreases in weaning weight and the number of piglets weaned, decreases in the farrowing rate and decline in body condition (Boulot *et al.,* 2008; Foxcroft, 2008).

During pregnancy, 20% to 40% of the available energy and amino acids are allocated for optimal growth of the foetuses, with this increasing as the sow approaches parturition. The remaining energy and amino acids (60–80%) are used for the maintenance of normal metabolism (Ball *et al*., 2008), growth of the sow to maturity and the development of reserves for mobilization during the subsequent lactation (Noblet & Etienne, 1987).

Lactation typically only lasts for 21–28 days but has a far greater impact on metabolism than the 114 day gestation period, having a greater impact on the metabolism of the sow than any other healthy physiological

state. The nutrient requirements of the sow during lactation are difficult to determine because of various influencing factors such as voluntary feed intake, body weight loss, composition of and milk production (Ball *et al.,* 2008).

The essential nutrients for pigs include energy, protein and amino acids, carbohydrates, fats, vitamins, minerals and water.

1.3.1 Energy

Energy plays a fundamental role in all life processes, which includes the production of milk, maintenance of blood pressure and muscle tone, the action of the heart, transmission of nerve impulses, protein and fat synthesis and reabsorption in the kidneys (Ensminger & Parker, 1984). The energy requirement of the pig is determined by its growth rate, weight, reproductive stage and maintenance requirements (Muirhead & Alexander, 1997). The energy content of the ration is the primary determinant of the performance of the pig and the most expensive component. In a balanced feed ration, the energy concentration of the feed plays a major role in determining the feed intake. The energy density of the diet regulates the daily feed intake and therefore the total energy intake stays relatively constant across different diets (Noblet & Van Milgen, 2004).

Not all the energy ingested by the pig is available for production and growth. The amount of energy in a diet is therefore usually expressed as either the digestible energy (DE) or the metabolisable energy (ME) (Muirhead & Alexander, 1997). The digestible energy content of a feed or feed ingredient refers to the energy that is absorbed after the energy excreted in the faeces of the pig (Van Milgen, 2006). The simplified schematic breakdown of the energy components is given in Figure 1 below.

The ME content of a diet is the DE minus the urinary and gaseous losses. The amount of energy lost in the urine is dependent on the amount of nitrogen in the urine. At a physiological age at which the amount of nitrogen in the body is stable, the urinary nitrogen content will be primarily dependent on the crude protein content of the diet (Noblet & Henry, 1993). When an animal is fasted and its ME intake is zero the energy retention is negative, therefore the animal uses its own body reserves to provide energy for the maintenance of essential bodily functions. This energy will leave the body as heat. However, when the energy retention is zero and the ME intake increases to a level that is sufficient the animal will utilise this additional energy for maintenance. A further increase in the ME intake will allow the animal to begin to retain energy, either as body tissues or production products (McDonald *et al*., 2002).

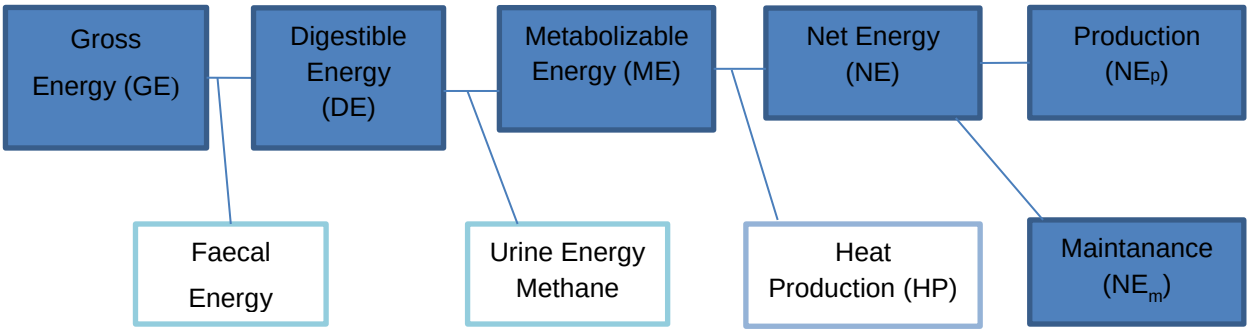

Figure 1: A simplified schematic breakdown of the energy components in a feed ingredient (Visser, 2014)

The net energy (NE) content of a feed is the ME minus the heat production (HP) with the latter including the metabolic usage of the ME and the energy cost of some physical activities, ingestion and digestion. The net energy content can be considered as the most accurate energy value for pigs (Visser, 2014). The NE system is the only system in which the dietary energy content and the energy requirements are expressed on the same basis (Noblet, 2007).

Energy is stored by the pig in products such as body fat, muscle and milk. The energy content of these products is largely contained in protein and fat, although milk contains greater proportion carbohydrates. The efficiency with which ME is utilised for production depends mainly on the metabolic pathways involved in the synthesis of protein and fat from absorbed nutrients and their energetic efficiencies (McDonald et al., 2002).

Energy intake is the main driver of protein deposition until a plateau is reached, after which further intake will result in fat deposition (Ensminger & Parker, 1984). Protein deposition is the result of two processes, the synthesis of proteins and their breakdown. In most bodily tissues, proteins are continuously broken down and resynthesized by reactions that generate heat. This process reduces the calorific efficiency of protein deposition. The protein synthesis process is dependent on the activation of amino acids, initiation of chain formation and chain elongation and termination, all of which have an energy cost. When in excess, amino acids may be utilised as a source of energy (McDonald *et al*., 2002). In addition, when dietary energy is deficient, amino acids are oxidised and utilised as an energy source (Wang & Fuller, 1989). One of the consequences of the catabolism of amino acids is the production of ammonia. The majority of ammonia produced is excreted by the body as urea, with some being used for trans-amination during amino acid synthesis. The energy required for urea synthesis is more than the energy obtained by the oxidation of the carbon skeleton of the amino acid (McDonald *et al.,* 2002).

1.3.2 Protein and amino acids

Pigs require a balanced feed that contains sufficient amounts of all nutrients, including energy and protein (Sauer & Ozimek, 1986a). Proteins always contain hydrogen, carbon, oxygen and nitrogen; and in addition sometimes sulphur (Ensminger and Parker, 1984). Amino acids are compounds that are joined together in different combinations to form the different proteins required by the body (Muirhead & Alexander, 1997).

In the intestinal tract proteins are broken down into individual amino acids, which are absorbed into the blood stream and transported around the body. The major roles of amino acids are in the production of muscle proteins, haemoglobin, digestive enzymes, gamma globulins, hormones and milk proteins (Wu, 2009).

Plants and many microorganisms have the ability to synthesise protein from simple nitrogenous compounds such as nitrates. Animals do not have this ability and must therefore have a dietary source of amino acids in order to build up body proteins. Non-essential amino acids can be synthesized de novo by the animal and some amino acids can be produced from others by transamination (Wu et al., 2013). However there are a number of amino acids for which the carbon skeletons cannot be synthesized by the body. These amino acids are referred to as essential or indispensable amino acids, and have to be supplemented in the diet. The essential amino acids for pigs are lysine, methionine, cysteine, threonine, tryptophan, valine, histidine, isoleucine, phenylalanine, tyrosine and leucine (McDonald *et al.,* 2002). The classification of the different essential amino acids is presented in Table 1.1 below.

Table 1.1 Chemical structure of the essential amino acids (adapted from McDonald *et al*., 2002)

Aromatic and heterocyclic amino acids	Basic Amino acids	Monoamino acids-monocarboxylic acids	Sulphur-containing amino acids
Phenylalanine, Tyrosine, Tryptophan	Histidine, Lysine	Isoleucine, Leucine, Threonine, Valine	Cysteine, Methionine

Good quality protein and amino acid availability is particularly important in pig nutrition during periods of management change, stress and immune challenge. The protein quality of the feed is a reflection of the availability and balance of the essential amino acids. The ideal protein concept refers to the balance in which amino acids are required for body protein accretion and maintenance (ARC, 1981; Wang & Fuller, 1989). This involves having the correct balance of essential and non-essential amino acids. High quality proteins contain all the essential amino acids at adequate levels while poor quality proteins are deficient in one or several amino acids (Sauer & Ozimek, 1986b). Minor changes in the concentrations of one or more amino acids may increase the amounts of others required to sustain growth rates (McDonald *et al*., 2002). The closer the amino acid balance of the ration is to the requirements of the pig, the less protein is wasted and therefore less nitrogen is excreted in the urine.

In the ideal protein concept, the specific requirements for essential amino acids are expressed relative to the lysine content (Jongbloed & Lenis, 1992). In maize-soybean meal based nursery pig diets lysine is the first limiting amino acid (Lewis *et al*., 1980), followed by methionine and cysteine, and then threonine, tryptophan, leucine and valine (Mavromichalis *et al.,* 1998). The ideal amino acid balance differs for the different production stages of the pig. This is due to differences in the composition of the proteins synthesised for maintenance, growth of lean tissue, pregnancy and lactation (McDonald *et al.,* 2002).

The requirements for additional amino acids can be met by using more protein-rich feedstuffs or amino acids in pure or crystalline form (Sauer & Ozimek, 1986b). Amino acid requirements are expressed as a percentage

of the diet but the DE content of the feed determines the voluntary feed intake. Amino acid intake is affected by the DE content of the diet. Excess energy can be stored in the form of fat but excess protein cannot be stored. The unused nitrogen fraction is discarded as urea and the carbon fraction is utilised as an energy source (Ensminger & Parker, 1984).

1.3.3 Carbohydrates

Carbohydrates are composed of carbon, hydrogen and oxygen. The sugar molecules range from simple sugar molecules (monosaccharides) with between three to seven carbon atoms to combinations of two, three or four molucules (di-, tri, and tetrasaccharides) and finally complex polymers of molecules (polysaccharides). Monosaccharides include glucose, fructose, arabinose, xylose and ribose, disaccharides include sucrose, maltose and lactose and the polysaccharides include starch, hemicellulose and cellulose.

Lignin is closely associated with this group but is not a carbohydrate. In the animal, lignin has a high resistance to chemical degradation and makes plant fibres inaccessible by enzymes for digestion. There are strong chemical bonds between lignin, cell wall proteins and many plant polysaccharides, which render these compounds unavailable for digestion (McDonald *et al*., 2002).

Non-starch polysaccharides (NSP) consist of β-glucans, cellulose, hemicellulose and pectin (Souffrant, 2001). The NSP contain both soluble and insoluble fractions and is the primary energy source for microbial fermentation in the large intestine of the pig (Knudsen *et al*., 1991; Knudsen, 1997). In the anterior part of the small intestine, there is an absence of cell-wall degrading enzymes and a low density of microorganisms. This enables the dietary fibre (DF) to stay more or less intact when arriving in the hindgut where it then degraded to a variable extent by a diversified microbial population (Fonty & Gouet, 1989).

Carbohydrates constitute a large portion of the pig's ration and serve as a source of heat and energy in the body. They are the primary source of energy and are responsible for at least 50% of the cost of the ration. Carbohydrates affect the function of the gastrointestinal tract and the digestion process. Surplus carbohydrates are transformed into fat and stored.

1.3.4 Fats

The function of fat is to serve as a source of heat and energy, and energy storage within the body. Fats and fat-like substances contain hydrogen, carbon and oxygen, similar to the carbohydrates. However, fats contain a larger proportion of hydrogen and carbon and have a lower heat increment. In pigs, the fatty acid composition of the dietary fat determines the fatty acid composition of the carcass fat, and the consumption of unsaturated fats could thus lead to the deposition of undesirable soft carcass fat (Ensminger & Parker, 1984).

1.3.5 Phytogenic Feed additives

In the livestock industry, plant-derived products are used as feed additives in order to improve production. Research on phytogenic feed additives has increased in recent years because of the ban on most antibiotic feed additives within the European Union in 1999, which was due to concerns about the development of

antibiotic-resistant pathogenic bacteria. There are a vast variety of phytogenic feed additives available, which includes spices, herbs, essential oils and oleoresins (substances prepared using solvent extraction processes). The active ingredients and levels thereof in these additives may differ substantially according to season, plant part (seed, root or leaf), harvesting season and geographical region. Another contributing factor is the mode of processing, which can also modify the associated compounds and active substances in the final product (Windisch *et al.,* 2008).

1.3.6 Fenugreek

Fenugreek (*Trigonella foenum-graceum*) is a member of the leguminosae family (Hamden *et al.,* 2010). This annual plant is both a medicinal and culinary herb which has been used for centuries and is mainly cultivated in Northern Africa, Southern Asia and India (Sauvaire *et al.,* 1991; Shim *et al.,* 2008). The medicinal uses of fenugreek for humans vary from wound healing, reducing blood sugar and cholesterol and promoting lactation. Both the leaves and seeds of this herb have been utilised extensively to prepare powders and extracts for medicinal purposes.

Fenugreek plants grow to a height of 60 cm and the seeds mature in long pods (Smith, 2003); both the leaves and seeds are edible (Petit *et al.,* 1995). The leaves are used as green vegetables and provide a good source of numerous minerals and vitamins, especially choline. The seeds can be utilized as a spice and are bitter. The seeds have antibacterial and galactogogue properties and stimulate the digestive system (Srinivasan, 2006). Chemical analysis of the seeds indicates that they are a rich source of protein, mucilage, non-starch polysaccharides and saponins (Rao & Sharma, 1987). Saponins are converted in the gastrointestinal tract to sapogenins, which may be responsible for lowering cholesterol levels (Smith, 2003). Structurally they consist of an aglycone nucleus with one or more carbohydrate side chains. Saponins are known to improve immune function (Ilsley *et al.,* 2005).

The medicinal value of fenugreek is mainly due to three important chemical constituents, namely galactomannans, isoleucine and steroidal sapogenins. These work in a synergistic manner to produce health benefits (Acharya *et al.,* 2006). The seeds also contain 50% fibre (20% insoluble and 30% soluble), which could have a secondary hypoglycaemic effect by reducing the rate of postprandial glucose absorption (Smith, 2003).

Dioscin, a steroid saponin, is a component of fenugreek and has a structure similar to that of oestrogen (Muraki *et al.,* 2011). Dioscin stimulates the production of growth hormone by binding to the pituitary cells (Hwang *et al.,* 2014). Growth hormone has a galactopoietic effect and could be involved in the mechanism by which fenugreek stimulates milk production (Alamer & Basiouni, 2005). Fenugreek can have a normalizing effect on the progesterone action of the pituitary gland and therefore stimulate prolactin in lactating mothers (Behera *et al.,* 2013). However, the effect of fenugreek on milk yield is still unclear and further research is needed to determine the mechanism of action (Al-Shaikh *et al.,* 1999).

1.3.7 Production parameters

The reproductive performance of sows can be influenced by dramatic changes in body weight or extreme body conditions such as emaciation or obesity. The aim of a sow feeding strategy must be to maintain body reserves without severe changes. During lactation the sow must be fed to minimise weight loss, and during successive gestations the sow must gain weight to enable growth to maturity (Noblet *et al*., 1990). Another important factor is to conserve the sow's body tissue reserves throughout her productive life, which will increase herd productivity (Young *et al.,* 2004). Sterning *et al.* (1997) noted that sows with a large relative weight loss during lactation had a higher incidence of reduced feed intake and seemed to be in a higher catabolic state in late lactation than those that only lost a small amount of weight.

In pig production, the farmer is faced with numerous challenges. One of these challenges is piglet mortalities, particularly in new-born piglets (Krakowski *et al.,* 2002). The neonatal piglet has very limited energy reserves and is very dependent on adequate colostrum intake. The colostrum provides energy for the maintenance of body temperature and normal physiological functions (Noblet *et al.,* 1997). It also transfers immunity from the sow to the piglet (Devillers *et al.,* 2011). The saponins in fenugreek are known to improve immune function. Ilsley *et al.* (2005) found that weaned piglets on saponin-supplemented diets had improved immunoglobulin (IgG) levels; suggesting that they may have had better immunity. The highest risk of mortality is usually during the first three days of life (Tuchscherer *et al.,* 2000), therefore colostrum intake is a crucial factor in the survival of the neonatal piglet and insufficient intake can result in mortality.

Higher birth weights ensure better average daily gain over the suckling, post weaning and growing periods (Quiniou *et al.,* 2002). Increased milk yields and functional teats are associated with heavier piglets at weaning; therefore the milk production of the sow is a very important factor (Auldist *et al.,* 2000). Weaning weight has a significant effect on the subsequent growth of the piglets (Wolter *et al.,* 2002), with piglets with high weaning weights having better average daily gains and average daily feed intakes than lighter piglets (Magowan *et al.,* 2011). This results in improved feed conversion ratios and allows slaughtering at an earlier age. Feed conversion ratio is an important measurement of profitability for the producer (Edwards *et al.,* 1989), and small improvements have a significant effect on the success of the operation.

The main benefits from fenugreek would be the galactopoietic effect on sows during lactation and the immune-stimulating activities. This could increase weaned litter weight and decrease piglet pre-wean mortality, however the mechanism of action still needs to be determined.

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Chapter 2

The effects of feeding fenugreek as a feed additive to sows in gestation and lactation on subsequent birth and litter weights

Abstract

In the livestock industry, plant-derived products are used as feed additives in order to improve production. The medicinal uses of fenugreek vary from wound healing to reducing blood sugar and cholesterol and promoting lactation. The effect of fenugreek on milk yield in the pig is still unclear and further research is needed to determine its mechanism of action. This trial was conducted using 120 multiparous sows from 85 days of gestation until the piglets were weaned at 28 days old. The effects of fenugreek on the production parameters of the sows and piglets were measured. The sows were housed in pens during gestation and individual farrowing crates during lactation. Two commercial Fenugreek products, Nutrifen® and Nutrifen Plus®, were fed to the sows during the last trimester of gestation and during lactation. The treatments were: 1) control (CON), with no fenugreek supplementation: 2) sows supplemented with 0.2% Nutrifen ®; 3) sows supplemented with 0.2% Nutrifen Plus®. The fenugreek treatments had a significant effect on the back fat thickness (mm) of the sows at farrowing, with both Nutrifen® and Nutrifen Plus® decreasing back fat thickness. The fenugreek treatments did not significantly influence the number of piglets born alive, the number of stillborn piglets, the number of mummified piglets, the litter birth weight (kg), the pre-weaning mortality (%), the piglets weaned per sow, the litter weaning weight (kg), the back fat thickness (mm) of the sows at weaning or the total feed intake during lactation (kg). In this investigation under these specific commercial conditions, the use of the two commercial fenugreek products had no clear advantage over the normal commercial diets fed to sows.

Keywords: fenugreek; lactation; production parameters

2.1 Introduction

During the first two trimesters of gestation there is hardly any quantitative growth of the mammary glands in the sow, with almost all development taking place in the last trimester (around 85 days of gestation) (Sørensen *et al*., 2002). The development and growth of the mammary glands plays a very important role in milk production and the amount of mammary tissue will likely determine the volume of milk produced (Farmer & Sørensen, 2001). The period just before farrowing is crucial for determining the number of mammary cells and the piglet birth weight. If the sow's nutritional requirements are not met, it could reduce the number of mammary cells and the piglets' birth weights. The milk production of the sow is considered the first limiting factor for the pre-weaning growth of the piglets until the piglets receive creep feed, the sow's milk is the only source of energy for the young piglets. Therefore, for optimal growth the sow must have high milk production (Farmer *et al.,* 2000).

The use of fenugreek as a galactogogue in humans is reported as far back as 1945, with women showing an increase in milk production 24 – 72 hours after the consumption of fenugreek (Gabay, 2002). As the case for many herbal products, the dose necessary for a galactogogic effect is still unclear (Zuppa et al., 2010). However, Al-Shaikh et al., (1999) reported in increase in milky yield in dairy goats when supplemented with 25 % fenugreek concentrate. In a study done Hossain et al. (2015), sows supplemented with 0.1 % and 0.2 % fenugreek seed extract during lactation weaned had higher weaning weights when compared to the control group, which could indicate an effect on milk production. Dioscin, a component of fenugreek, is a steroid saponin with a structure similar to that of oestrogen (Muraki *et al.,* 2011). It stimulates the production of growth hormone by binding to the pituitary cells (Hwang *et al.,* 2014). Growth hormone, in turn, has a galactopoietic effect, which could provide an explanation for the mechanism of action of fenugreek (Alamer & Basiouni, 2005).

Nutrition has a considerable effect on the circulating growth hormone levels in pigs (Brameld, 1997). Maternal growth hormone cannot cross the placental membranes and be transferred to the piglets; however, high levels of insulin-like growth factors (IGF) may enhance the transfer of nutrients across the placenta. This can increase the levels of insulin-like growth factors in the foetus and consequently promote foetal growth rate (Lassarre *et al.,* 1991). In a study by Gatford *et al.* (2000), porcine growth hormone was administered to restricted-fed sows during early- to mid-gestation and this was found to increase foetal body weight at day 51 of pregnancy. When growth hormone is administered to sows during gestation it can stimulate placental growth, increase nutrient availability to the foetus/embryo and cause long- and short-term changes in the IGF-I serum concentrations in the piglets. Foetal growth can be accelerated by maternal growth hormone treatment but the growth achieved during early- and mid-gestation cannot be maintained until birth. However, Rehfeldt (2005) administered porcine growth hormone to sows during late gestation, which resulted in heavier piglets at birth.

It would be interesting to see whether there is a link between fenugreek supplementation to the gestating and lactating sow on sow reproductive performance and litter parameters. Thus, the following hypothesis was tested:

H0: Fenugreek as a feed additive will not affect sow reproductive performance and litter parameters

H1: Fenugreek as a feed additive will affect sow reproductive performance and litter parameters

2.2 Materials and Methods

2.2.1 Ethical clearance for animal use

This study complied with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008, and was completed with ethical clearance from the Stellenbosch University Care and Use Committee (SU ACUC), reference number: SU-ACUD15-00056.

2.2.2 Animals used in the study

The pigs used in this study were obtained from a commercial farm in the Western Cape Province of South Africa. The farm uses only PIC hybrid lines, which are produced by crosses between the Landrace, Large

White and White Duroc. They utilise a semen-only program and artificially inseminate with the PIC line 337 boar to produce the terminal offspring and the PIC line 2 for the maternal offspring.

The study used 120 sows and their 1480 piglets. All sows were moved to the dry sow house within the first five days of gestation, where they were housed in individual crates. The sows received a standard commercial dry sow diet during this time. The trial was done on two groups of 60 sows each, with varying farrowing statuses present within each group (gilt to 8th parity). Sows entered the trial at 85 days of gestation and on day 105 of gestation the sows were moved to the farrowing house. The sows were housed in an environmentally controlled house in individual farrowing crates. All sows received a commercial lactation diet until the piglets were weaned at 28 days old, which was the end of the trial. In the farrowing house the piglets were fed a commercial piglet creep diet from day 10 until weaning.

2.2.3 Feed characteristics

The feed used in the trial was a commercial feed made by a commercial feed company. The sows received 3 kg of dry sow feed per day in the dry sow house during gestation and 2–8 kg of lactation feed per day in the farrowing house. All the sows had access to fresh and clean water daily. The nutritional composition and formulation of the dry sow feed is presented in Table 2.1 and Table **2.2**, and the nutritional composition and formulation of the lactation feed is presented in Table 2.3 and **Error! Reference source not found.**.

Table 2.1 Nutritional composition of dry sow treatment diets

Nutritional Composition (%)	Treatment 1	Treatment 2	Treatment 3
Protein	11.96	12.54	13.08
Crude Fat	2.15	2.12	2.96
Crude Fibre	8.65	7.91	7.71
Ash	5.42	4.98	5.18
Moisture	10.5	11.47	10.48
Calcium	0.91	0.65	0.81
Sodium	0.19	0.17	0.21
Magnesium	0.25	0.17	0.23
Phosphorus	0.45	0.41	0.45
Potassium	0.74	0.82	0.72

Table 2.2 Raw material composition of dry sow treatment diets

Ingredient (% as fed)	Treatment 1 Control	Treatment 2	Treatment 3
Soya bean Hulls	5.00	5.00	5.00
Wheat Bran	22.77	22.8	22.8
Oat Bran	2.23	2.2	2.20
Maize	54.34	54.34	54.34
Lupins	8.00	8.00	8.00
Soya Oil Cake (47%)	3.00	3.00	3.00
Sunflower Oil Cake	1.33	1.37	1.37
Lysine	0.07	0.07	0.07
Threonine	0.003	0.003	0.003
MonoCalcium Phosphate	0.075	0.075	0.075
Limestone Fine	1.467	1.467	1.467
Salt Fine	0.548	0.503	0.503
Panbonis Plus	0.10	0.10	0.10
Phytase	0.05	0.05	0.05
Vitaroma	0.025	0.025	0.025
Mycofix Select	0.10	0.10	0.10
Acid Buff	0.40	0.40	0.40
Nutrifen®	0.00	0.20	0.00
Nutrifen Plus®	0.00	0.00	0.20
Premix - Lactation	0.30	0.30	0.30

Table 2.3 Nutritional composition of lactation treatment diets

Nutritional Composition (%)	Treatment 1	Treatment 2	Treatment 3
Protein	15.37	14.97	15.63
Crude Fat	4.23	4.19	3.95
Crude Fibre	5.87	5.86	5.57
Ash	5.62	5.99	5.49
Moisture	11.40	11.75	12.05
Calcium	0.93	0.89	0.95
Sodium	0.22	0.22	0.22
Magnesium	0.20	0.67	0.21
Phosphorus	0.47	0.47	0.47
Potassium	0.81	0.82	0.89

Table 2.4 Raw material composition of lactation treatment diets

Ingredient (% as fed)	Treatment 1 Control	Treatment 2	Treatment 3
Lucerne Meal	3.00	3.00	3.00
Wheat Bran	17.27	16.83	16.83
Maize	53.29	53.29	53.29
Soya Oil	0.40	0.43	0.43
Molasses Syrup	3.00	3.00	3.00
Fishmeal (65%)	2.00	2.00	2.00
Lupins	8.00	8.00	8.00
Soya Oil Cake (47%)	6.53	6.73	6.73
Full Fat Soya	3.00	3.00	3.00
Lysine	0.25	0.23	0.23
Methionine	0.06	0.06	0.06
Threonine	0.06	0.06	0.06
Tryptophan	0.02	0.02	0.02
MonoCalcium Phosphate	0.32	0.32	0.32
Limestone Fine	1.77	1.77	1.77
Salt Fine	0.47	0.47	0.47
Panbonis Plus	0.10	0.10	0.10
Phytase	0.05	0.05	0.05
Sucram	0.01	0.01	0.01
Vitaroma	0.025	0.025	0.025
Mycofix Select	0.10	0.10	0.10
Nutrifen®	0.00	0.20	0.00
Nutrifen Plus®	0.00	0.00	0.20
Premix - Lactation	0.30	0.30	0.30

2.2.4 Treatments

Two commercial fenugreek products, Nutrifen® and Nutrifen Plus®, were used. Both products contain cotyledon concentrate but are formulated differently. The sows from each treatment were placed together in groups of 20 per treatment per week and were identified by different coloured crates to ensure that each animal got the appropriate treatment.

The composition of the two products is as follows:

Nutrifen®:

- Fenugreek cotyledon concentrate (Trigonella foenum-graecum)
  Active compound: 30mg/g diosgenin

NutrifenPlus®:

- Fenugreek cotyledon concentrate (Trigonella foenum-graecum)
- Fennel seed (Foeniculum vulgare)
- Kelp (Laminariales)

- Saw Palmetto berries (Serenoa Repens)
- Brown, MSM (natural source Methylsulfonylmethane)
- White distilled vinegar powder

Active compound: 21.6 mg/g diosgenin

2.2.5 Dietary treatments

2.2.6 Gestation

The control diet was the standard commercial ration used on the farm and was formulated according to PIC nutritional recommendations. The trial diets consisted of the control diet supplemented with either 0.2% Nutrifen® or 0.2% Nutrifen Plus®. All the sows received 3 kg of feed per day.

A total of 60 multiparous sows were used per week. The sows were selected according to parity and divided into three groups of 20 sows each for the three different treatments, with each treatment containing an approximate equal number of sows of each parity. The treatments were repeated the following week, therefore the total number of sows used was 120 for the trial, with 40 sows per treatment group. The layout and schedule for the trial is shown in **Error! Reference source not found.**.

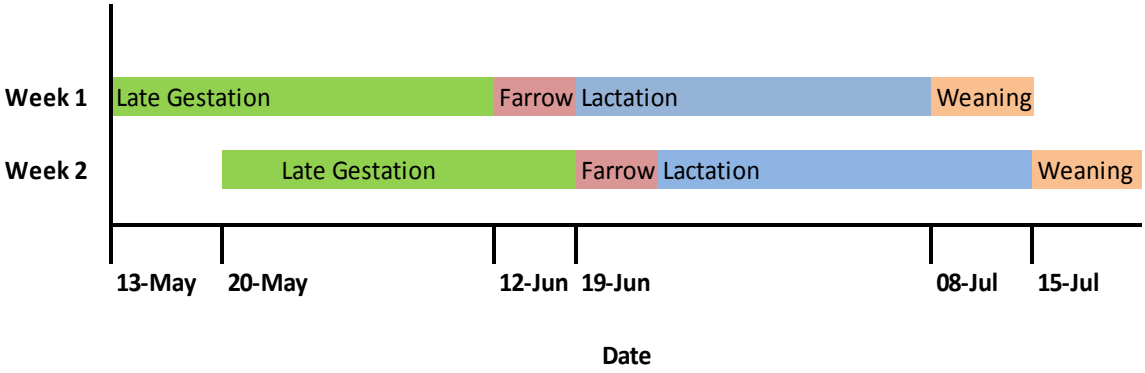

Figure 2.1 Experimental design and schedule of the trial

Treatment 1 was the control group, with no supplementation at any stage of the production process whilst treatment 2 was supplemented with 0.2% Nutrifen® from day 85 until day 105 of gestation. Treatment 3 was supplemented with 0.2% Nutrifen Plus® from day 85 until day 105 of gestation. The whole process was repeated in the second week. The parity distribution of the different treatments is presented in Table 2.5. The period and treatment combinations of the sows are presented in Table 2.6.

Table 2.5 Parity distribution for the different treatment groups

Parity	0	1	2	3	4	5	6	7	8
Treatment 1	9	5	4	6	5	3	2	3	0
Treatment 2	8	6	3	6	4	3	4	4	1
Treatment 3	11	6	4	5	4	3	3	2	0

Table 2.6 The period and treatment combinations of sows

Period	Days	Treatment 1	Treatment 2	Treatment 3
Gestation				
Day 85-105	21	Control	Nutrifen®	Nutrifen Plus®
Lactation				
1 week before	7	Control	Nutrifen®	Nutrifen Plus®
Week 1	7	Control	Nutrifen®	Nutrifen Plus®
Week 2	7	Control	Nutrifen®	Nutrifen Plus®
Week 3	7	Control	Nutrifen®	Nutrifen Plus®
Week 4	7	Control	Nutrifen®	Nutrifen Plus®

2.2.7 Lactation

On day 105 of gestation, all the sows were moved to the farrowing house. Before farrowing, the sows were fed 2 kg of lactation feed per day. After farrowing, a step-up feeding program was followed in order to stimulate feed intake, and continued until day seven of lactation. Starting at 2 kg of lactation feed at day of farrow, each day there was a 1 kg feed increase until day seven, after which the feed provision plateaued at a maximum amount of 8 kg per day. After day eight, the sows were fed according to individual voluntary feed intake until day 28 of lactation.

The treatments continued as applied in the dry sow house. Treatment 1 was fed the commercial lactation diet, Treatment 2 the control diet supplemented with 0.2% Nutrifen® and Treatment 3 the control diet supplemented with 0.2% Nutrifen Plus®.

Cross-fostering took take place within the first day of farrowing between gilts/sows in the same treatment group in order to have a standardized litter size of 12 piglets per litter.

2.2.8 Duration of the trial

The trial was conducted for two months from May to July 2015 on a commercial farm in the Western Cape, which is during the winter period.

2.2.9 Weighing of the piglets

After farrowing was complete the whole litter was weighed (MICRO T7E, B & R Scale Services) together to provide the litter birth weight. At weaning, the weaning weight of the litter was also recorded using a crate and a hanging scale (ADAM® AE402, B & R Scale Services). All the piglets were placed in the crate and the weight was recorded.

2.2.10 Back fat measurement

Back fat measurements were performed using a Renco Lean-Meater® SERIES 12 ultrasonic instrument. The point of measurement was marked with a livestock marker to ensure that the subsequent measurement was done on the same point. Back fat thickness was determined by measuring the fat depth at the last rib of the sow and 6.5 cm on both sides from the midline and calculating the mean between the two measurements.

2.3 Statistical Analysis

For analysing sow and litter performance, ANOVA was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 2012), with the following dependant variables: born alive, born dead, mummies, birth weight, number weaned, litter weight, piglet weight, back fat at farrowing, back fat at weaning and feed intake. All of the variables were tested for normality and if normal were log-transformed. The parity number of the sows was taken in consideration as a co-variable. The differences in the data were deemed significant when $P \leq 0.05$ and trends were noted when $0.05 < P < 0.10$. The variability in the data was expressed as the pooled standard error of the mean (SEM).

2.4 Results and Discussion

In commercial pig farming, herd management is key for achieving production parameter goals and can be achieved by focusing on certain parameters. There are interrelationships between factors that need to be known in order to utilise their influence on the final productivity of the system (Plà, 2007). These factors include the number of piglets born alive, still born or mummified, the litter birth weight, the number of piglets weaned per sow, the weaned litter weight, the piglets' weaning weights, the back fat loss during lactation and the feed intake per sow per day during lactation.

2.4.1 Born Alive

The number of piglets born per sow can be influenced by uterine capacity, embryonic survival rate and ovulation rate (Tummaruk *et al.,* 2001). This production parameter is an important factor for measuring sow productivity (Rodriguez-Zas *et al.,* 2003) as an improvement in this regard will enable the producer to produce

more piglets per sow per year and thereby increase profitability. The effect of the different treatments on the reproductive and growth performance of sows and their piglets is presented in Table 2.7.

There was a treatment effect (P = 0.026) on the number of piglets born alive per sow. The control group had the highest number, with an average of 13.57 piglets born alive per sow, followed by the Nutrifen Plus® group, with 13.38 piglets. The Nutrifen group® had the poorest performance with 12.08 piglets born alive per sow on average. The control group and the Nutrifen Plus® group did not differ significantly from each other but both groups differed significantly from the Nutrifen® group. It is possible that these differences may reflect variation in factors such as genetics, nutrition, lactation length, inseminator efficiency, pregnancy problems and disease rather than the effect of the nutritional treatment, despite the efforts made to standardise these factors across the treatment groups (Lawlor & Lynch, 2007).

Table 2.7 The effect of Nutrifen® and Nutifen Plus® supplementation during gestation (from 85 days until farrowing) and lactation (from farrowing until weaning at 28 days) on the reproductive and growth performance of sows and their piglets

Production Parameters	Treatments			SEM	P-value
	Control	Nutrifen®	Nutrifen Plus®		
Born Alive	13.57[a]	12.08[b]	13.38[a]	0.23	0.026
Still Born	0.51	0.48	0.61	0.05	0.543
Mummy	0.16	0.28	0.21	0.04	0.464
Birth Litter Weight (kg)	18.74	17.26	19.09	0.34	0.489
Pre-wean Mortality (%)	8.58	7.89	5.50	1.20	0.188
Piglets Weaned	11.78	11.73	11.67	0.12	0.948
Weaned Litter Weight (kg)	85.54	83.59	86.44	1.13	0.592
Piglet Weaning Weight (kg)	7.29	7.16	7.42	0.084	0.489
Back fat Farrowing (mm)	19.68[a]	18.21[a]	17.36[b]	0.34	0.025
Back fat Weaning(mm)	15.56	14.50	13.94	0.28	0.066
Total Feed Intake (kg)	169.33	175.77	176.88	2.35	0.436
Feed Intake per day (kg)	5.05	5.29	5.30	0.07	0.314

[a,b,c] Means in the same row with different superscripts differ (P < 0.05)

There was no difference between the two weeks in the number of piglets born alive (P = 0.055); however, it should be noted that the first group of sows that farrowed had a higher number of piglets born alive (13.47) than the second group (12.55). Although this was not statistically significant, it could have an effect on other factors, such as the litter birth weight, piglet pre-weaning mortality and number of piglets weaned per sow.

Over the years, the selection for more piglets born alive per sow has resulted in an increasing number of piglets with low birth weights, which have a lower chance of survival and poorer weight gain (Van der Lende & De Jager, 1991; Milligan *et al.,* 2002).

2.4.2 Stillbirths

There was no difference between the treatments in the number of piglets born dead (P = 0.5433); however, the parity of the sow seemed to have an effect. There was a non-significant incline in the number of piglets born dead with an increase in parity from zero to eight (P = 0.2719).

Around 30% of stillbirths are caused by pathogenic agents, which can be specific or non-specific uterine pathogens. Viruses, protozoa and bacteria can infect the uterine environment via the haematogenous route, through the vagina during natural service or through contamination of the semen. Non-specific pathogens are mainly bacteria that enter the uterus and these can be introduced through insemination or ascending infections (Vanroose *et al.,* 2000). Other factors that are related to stillbirths include the positioning in the birth order, piglet haemoglobin levels, the length of the birth intervals between piglets and the condition of the umbilical cord, to name but a few (Zaleski & Hacker, 1993).

2.4.3 Mummies

The critical periods for foetal mortalities are between 35 and 40 days, 55 to 75 days and after 100 days of gestation. Prenatal mortalities are the highest in the first month of pregnancy (Ferguson *et al*., 2006). From 30–115 days of pregnancy the foetus is maturing and developing a skeletal system, and therefore if piglet death occurs during or after this period the foetus cannot be absorbed and a mummified piglet is present at parturition.

There are two main causes of mummified piglets. The first is infectious disease, such as Porcine Parvo virus, which is known to cause reproductive failure (Antonis *et al.,* 2006). Certain viruses can affect the foetus as early as 30 days of gestation and progressively spread during the remaining period of the pregnancy. After 70 days of gestation the piglets become immuno-competent and therefore can respond to infections (Muirhead & Alexander, 1997).

There was no significant effect of the fenugreek treatments (P = 0.4636) on the number of mummified piglets born. There was however a significant parity effect (P = 0.0278). The instance of mummified piglets was the highest in gilts and declined linearly with maternal age.

The second cause of mummified piglets, namely insufficient capacity in the womb, could explain the parity effect found in this trial, as gilts have less uterine capacity than older sows. Uterine capacity can be defined as the number of foetuses that can be carried to term. The effect of uterine capacity on litter size begins after 30 days of gestation, when nutrients and space become limited; resulting in competition between litter mates (Ford *et al.,* 2002).

2.4.4 Birth weight

Over the past decade, the prolificacy of sows has increased and this increase in litter size has led to a decrease in piglet weight because of limitations in uterine capacity (Madsen & Bee, 2015). Piglet birth weight can be considered the most important factor determining piglet survival (Roehe, 1999; Roehe & Kalm, 2000). Piglets with low birth weights have higher mortality rates and, if they survive until weaning, their post-weaning and subsequent growth is also negatively affected (Quiniou *et al*., 2002).

There was no significant treatment effect on birth weight (P = 0.0831), with fenugreek supplementation during the late gestation period not increasing the birth weight of the piglets. This supports the results reported by Hossain *et al*. (2015), who similarly did not find a significant effect on birth weight despite the significantly increased average daily gains and weaning weights of piglets that nursed from sows that received fenugreek treatments.

Mating group did have a significant effect on birth weight (P = 0.004). This is due to group one having a higher number of piglets born alive and therefore a higher litter birth weight, which is presented in Table 2.8.

Table 2.8 Piglets born alive and birth litter weight between the two different weeks

Parameter	Week		SEM	P Value
	1	2		
Piglets born alive	13.47	12.55	0.017	0.055
Birth litter weight	19.42	17.31	0.011	0.004

2.4.5 Pre-weaning piglet mortality

In the breeding herd, one of the main reasons for a loss in production is pre-weaning piglet mortality, which normally occurs within the first few days after farrowing (Pedersen *et al.,* 2006). In an efficient and well-managed breeding herd an average pre-weaning mortality of 5–8% should be maintained (Muirhead & Alexander, 1997).

There was no effect (P = 0.188) of the fenugreek treatments on the pre-weaning mortality. The control treatment had a pre-weaning mortality rate of 8.58%, Nutrifen® 7.89% and Nutrifen Plus® 5.50%. Interestingly, Ilsley *et al.* (2005), found that quillaja saponins improved the immune response of weaned piglets in commercial rearing facilities. However, the reduced mortality rates in the groups that received the fenugreek treatments did not result in more piglets being weaned per sow, most probably because the control group had the highest number of piglets born alive.

Damm *et al.* (2005) noted that the main causes of piglet mortality were crushed piglets and starved piglets. In this investigation (Table 2.9), there was no effect (P = 0.484) of the fenugreek treatments on the number of piglets crushed or that died of starvation (P = 0.451).

Table 2.9 The effect of Nutrifen® and Nutrifen® Plus supplementation to sows during late gestation and lactation on the number of piglet mortalities due to crushing or starvation and the total piglets born alive per sow

Reason	Treatment			SEM	P Value
	Control	Nutrifen®	Nutrifen Plus®		
Crushed	14	9	10	0.82	0.484
Starvation	29	28	18	0.82	0.451
Total mortalities	43	37	28	1.20	0.188
Total piglets born alive per sow	13.57[a]	12.08[b]	13.38[a]	0.23	0.026

a,b,c Means in the same row with different superscripts differ (P < 0.05)

There was a difference (P = 0.001) in the piglet mortality rate between the two mating groups of sows (Table 2.10). The first week had a pre-weaning mortality rate of 12.10%, and the second week 7.46%. This is consistent with reports in literature, as increases in litter size tend to increase the birth weight variation of the piglets and the parturition time of the sows (Dyck & Swierstra, 1987; Quesnel *et al.,* 2008; Weber *et al.,* 2009). The survival of perinatal piglets is dependent on a series of complex interactions between the sow, the piglet and the rearing environment (Milligan *et al.,* 2002). Crushing and starvation of piglets within the first week after farrowing are the predominant causes of piglet mortalities (Dyck & Swierstra, 1987; Marchant *et al.,* 2000; Damm *et al.,* 2005). Starvation and crushing are correlated because the starved piglets are more likely to get crushed. The newborn piglet has limited energy reserves and is prone to starvation. Piglets with low birth weights are more likely to be crushed because they are more susceptible to cold and therefore try to huddle close to the sow for warmth, as well as trying to stay close to the udder for milk (Weary *et al.,* 1996). Other causes of mortalities are euthanasia, congenital defects, exposure and disease (Dyck & Swierstra, 1987). Selection programs for piglets born alive which is the norm, rather than piglets weaned per sow can lead to decreases in the maternal ability of the sows (Lund *et al.,* 2002).

Table 2.10 The effect of mating batch on the number of piglet mortalities due to crushing or starvation and piglets born alive per litter

Reason	Week		SEM	P Value
	1	2		
Crushed	19	14	0.50	0.434
Starvation	58	18	0.48	0.001
Total Mortalities	77	31	1.20	0.001
Piglets born alive	13.47	12.55	0.017	0.055

2.4.6 Piglets weaned

During 2013 in the South African pig industry it was estimated that the average producer weaned ca. 23.5 piglets per sow per year, the above average producer 27 piglets per sow per year and the best producers more

than 28.5 piglets per sow per year. While the number of sows in South Africa has decreased in recent years the number of piglets weaned per sow per year has increased. This may be due to factors such as genetic improvements, the use of environmentally controlled houses, enhanced nutritional management, improved biosecurity and better overall management (Visser, 2014).

There was no treatment effect (P = 0.948) on the number of piglets weaned per sow. This is in agreement with Hossain *et al.* (2015), where supplementation with different fenugreek seed extract treatments during lactation had no significant effect on the number of piglets weaned per sow.

Parity had a significant effect (P = 0.007) on the number of piglets weaned per sow. Younger sows had the highest number of piglets weaned when compared to the older parities. This could be due to the gilt management system used on the farm. In order to stimulate optimum milk production during the sow's productive lifespan gilts are fostered 12 piglets to maximize udder development. Gilts receive priority relative to higher parity sows. Cross-fostering took place between gilts/sows within the same treatment in order to have a standardized litter size of 12 piglets.

The number of piglets weaned per sow per year is one of the most important production parameters in breeding units and commercial farms in South Africa. This production parameter influences the profitability of the farm because an improvement means that more pigs can be marketed with the same fixed/overhead costs. There are a minimum number of pigs that needs to be transferred to the nursery and finisher buildings in order to cover the basic unit costs. The excess piglets represent the profit, as their costs only include the creep feed, additional lactation feed for the sow and a minimal amount of variable costs (Visser, 2014).

2.4.7 Litter weaning weight and average piglet weight

As previously mentioned, many producers or breeding programs focus on the litter size of the sow as a selection standard instead of the litter weight and piglet weight as the latter are time consuming and costly to measure. One must be aware of the relationship and the possible negative effect of selection for litter size on piglet birth and weaning weights (Kaufmann *et al.,* 2000). Increases in litter size negatively affect piglet birth weight, and piglets with low birth weights have a lower viability and therefore a higher pre-weaning mortality, and reduced post-weaning growth (Roehe, 1999).

Under commercial conditions, variation in live weights, specifically within a weaning group (Mahan *et al.,* 1998), can have a negative effect on the profitability of the producer, especially in all-in, all-out systems. The light piglets at weaning require more days to reach market weight than their heavier littermates (Wolter & Ellis, 2001). In the past, the cost of variation was often overlooked as the continuous flow system allowed the producer to select only the market-ready pigs and the light pigs could stay in the pen longer until they reached the required weight. In the all-in, all-out system all the pigs of the same age group must leave the farm for market at the same time, so variation in live weight results in the producer being penalized for heavy pigs (>95 kg live weight) and lighter pigs (<70 kg live weight) in South Africa (Patience & Beaulieu, 2006).

There was no significant effect of the fenugreek treatments on the weaned litter weight (P = 0.592) or the piglet weight (P = 0.616). There are numerous factors that have an effect on the average daily gain from birth to

weaning. These factors include the litter, the sow, the farrowing and lactation environment and the piglet (Johansen *et al.,* 2004). Parity had an effect (P = 0.004) on the piglet weaning weight. The gilts produced the lightest piglets, with a linear increase in piglet weight with parity. This supports the finding of Koketsu & Dial (1997), who reported that first parity sows had the lowest weaning weights and litter sizes As a result of cross fostering the gilts had the highest number of piglets and the lowest milk yield per sow.

The sow must have the ability to produce enough milk to meet the requirements of the rapidly growing piglets. Piglets are born with relatively low energy reserves and deficient immune protection. The ingestion of colostrum, which is a source of energy and maternal antibodies, protects the piglet against pathogens until its own immune system has matured. This is critical for piglet's survival and the sow-piglet bond. The piglet needs energy for growth, physical activity and thermoregulation (Le Dividich *et al*., 2005).

2.4.8 Feed Intake

The amount of feed ingested during lactation has an effect on the subsequent reproductive performance of the sow (Koketsu *et al*., 1996). Suboptimal feed intake during lactation can reduce the rate of embryo survival in the following breeding cycle, increase the weaning to service interval and cause the sow to become catabolic (Baidoo *et al*., 1992; Yang *et al.,* 2000).

There was no treatment effect (P = 0.436) on the total feed intake of the sows during lactation. This finding contradicts the hypothesis that the steroidal saponins in fenugreek could increase appetite (Franki *et al.,* 2009; Liu *et al.,* 2012). Fenugreek is commonly used to add flavour to food therefore due to its aromatic properties it can have an effect on feed intake (Al-Habori & Raman, 1998)

The voluntary feed intake during lactation depends on the body condition of the sow at the end of gestation. Fat sows tend to eat less than lean sows during lactation, as was found by Revell *et al.* (1998), where sows that were fat at the beginning of lactation maintained their body condition throughout lactation, as did the lean sows. However, the fat sows consumed 30% less feed than the lean sows. There is a negative correlation between back fat thickness loss during lactation and feed intake during lactation (Dourmad, 1991).

2.4.9 Back fat thickness

Due to the economic pressure on current pig farmers, the evaluation and maintenance of the sow's body condition is very important in order to achieve desired production targets (Maes *et al*., 2004). There was a significant treatment effect (P = 0.025) on the back fat thickness at farrowing. The control group had the highest measurement of 19.68 mm, the Nutrifen® group had 18.21 mm and the Nutrifen Plus® group 17.36 mm. The back fat thickness loss of the sows during lactation is presented in Table 2.11. This can be due to the effect of the saponins in fenugreek, which affect the lipid metabolism. The fenugreek seeds have the potential to modulate the activity of certain enzymes including those associated with lipid metabolism and glucose (Raju et al., 2001) .Commercial producers use this measurement as an indication of body condition loss during lactation and adjust the gestation feeding curve according to the back fat measurement at weaning.

Table 2.11 Back fat thickness loss of the sows over the lactation period (farrowing until piglets weaned at 28 days)

	Treatment			SEM	P Value
Parameter	**Control**	**Nurtifen®**	**Nutrifen Plus®**		
Back fat loss	4.14	3.67	3.47	0.81	0.164

There was no significant effect of the fenugreek treatment on the loss of back fat during lactation (P = 0.164) or the back fat thickness at weaning (P = 0.066). There is a negative correlation between weight loss during lactation and subsequent reproductive performance (Thaker *et al*., 2005).

2.4.10 Limitations

As the trial was performed on a commercial farm the conditions were not ideal for trial purposes, particularly individual feed intake in the dry sow house. While the sows were housed in individual crates the feed was provided in a single long trough that supplied a number of different crates. It was therefore only possible to determine feed intake per treatment.

2.5 Conclusion

When used as a supplement Nutrifen® and Nutrifen® Plus did not have an effect on the reproductive performance of sows and litter parameters during lactation. Increased supplementation rates could be an option for future research.

There was an effect on the piglets born alive per sow but this could not have been a nutritional treatment effect but rather factors such as genetics, nutrition, lactation length, inseminator efficiency, pregnancy problems and disease, despite the efforts made to standardise these factors across the treatment groups.

There was also a significant effect of the fenugreek treatments on the back fat thickness of the sows at farrowing. The Nutrifen Plus® sows had the lowest back fat thickness followed by the Nutrifen® group. However, there was not a significant effect on the back fat thickness loss during lactation between the treatments. This is the more important factor as there is a negative correlation between weight loss during lactation and subsequent reproductive performance.

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Chapter 3

Fenugreek as a feed additive for gestating and lactating sows and the effects thereof on the blood profiles and mortalities of the piglets.

Abstract

Fenugreek has been used as both a medicinal and culinary herb for centuries. The saponins in fenugreek are known to stimulate humoral and cell-mediated immunity and exhibit various immune-stimulating effects. Literature on the immune-stimulating effect of fenugreek in pigs is limited and therefore there is a need to establish the correct application. This trial used 120 sows from the 85th day of gestation until the piglets were weaned at 28 days old, and evaluated the effects of fenugreek on the blood profiles of the sows and their piglets. Two commercial fenugreek products, Nutrifen® and Nutrifen Plus®, were used. The different treatments were: 1) control (CON), no fenugreek supplementation: 2) sows supplemented with 0.2% Nutrifen ®; 3) sows supplemented with 0.2% Nutrifen Plus®. The sows were housed in pens during gestation and in individual farrowing crates during lactation. For the blood analysis, only the parity three and four sows and their litters were used. At weaning, each whole litter was weighed and the piglet closest to the average weaning weight was used for blood sampling. The fenugreek treatments did not significantly influence the white blood cell count (WBCC), red blood cell count (RBCC), lymphocyte (LYM) and immunoglobulin G (IgG) levels of the sows or the piglets. It can be concluded that the use of these two commercial fenugreek products under these commercial conditions had no influence on the immune status of the sows or their piglets.

Keywords: blood profiles; fenugreek; IgG; lactation; LYM; RBCC; WBCC

3.1 Introduction

Continued human population growth, economic progress and urbanisation, and changes in consumers' preferences, have resulted in an increased demand for meat, eggs and milk. This has driven the rapid evolution of livestock systems (Udo *et al*., 2011). In order for producers to meet the growing demand, they have had to expand over the past decades and exploit the economy of scale. Producers who accomplished this were driven by technical progress and the global exchange of products and knowledge. However, their success is still influenced by their access to natural resources, technology, financing and markets, as well as personal mobility (Seré *et al*., 2008).

In South Africa, commercial (intensive) pig producers represent 86%, and communal pig producers 14% of the industry. Intensification can be referred to as the use of external resources to increase the quantitative and

qualitative output per animal. In practise, this entails applying modern technology and advanced management methods to increase the production per animal (Bebe *et al*., 2002). In intensive systems where large groups of animals are being kept together in a high-density situation, efforts should be made to prevent a disease from entering the farm. In addition, there should be control measures in place to reduce the impact and the spread of diseases already present on the farm. The implementation of such measures is referred to as biosecurity. Biosecurity can be classified into three groups: bio-exclusion, bio-containment and bio-management. Bio-exclusion includes practices aimed at preventing pathogens from entering the farm, bio-containment involves control measures that prevent existing pathogens from spreading to other farms and bio-management encompasses control measures that reduce the impact of existing endemic diseases on the farm (Visser, 2012).

Pigs are most susceptible to pathogens when newborn, relative to the other production stages. Immunologically the piglet is underdeveloped because of a lack of exposure to antigens and this is exacerbated by their physiological immaturity (Rooked & Bland, 2002). The primary reason for mortality in newborn piglets is an inadequate intake of colostrum, with suboptimal colostrum intake also potentially leading to infections and making the piglet more susceptible in the postnatal period and after weaning (Drew & Owen, 1988). The newborn piglet is dependent on the sow for immune protection because their immune system is naive. The piglet's immune system establishes during the period from farrowing to weaning.

The transfer of passive immunity from the sow to the piglet occurs postnatal via immunoglobulins and other bio-active peptides such as growth factors and cytokines in the colostrum and milk (Schanbacher *et al*., 1997). Immunoglobulins are specific antibodies found in blood, milk and particularly colostrum. There are three different types, namely immunoglobulin A, immunoglobulin G and immunoglobulin M (Muirhead & Alexander, 1997). The principle antibody isotope is immunoglobulin G, which is found in blood and extracellular fluid. This antibody controls the transmission of blood-borne and other pathogens between different body tissues (Litman *et al*., 1993).

The medicinal value of fenugreek is mainly attributed to three important chemical constituents, namely the galactomannans, trigonelline and steroidal saponins (Srinivasan, 2006). These constituents work synergistically to produce the health benefits observed (Acharya *et al*., 2006). Saponins are known to stimulate humoral and cell-mediated immunity and exhibit various immunostimulating effects (Bin-Hafeez *et al*., 2003 ; He *et al*., 2012). Hossain *et al.* (2015) supplemented lactating sows with fenugreek seed extract who showed increased serum IgG concentrations, as did their suckling piglets. This could indicate that the active compounds present in the fenugreek seed extract has the ability to increase the levels of IgG in sows and therefore increase the IgG levels of the piglets obtain via colostrum. Fenugreek seeds also have anti-oxidant properties, which enhance immunity (Yoo *et al*., 2008). Oxidative damage at the subcellular or cellular level is a major event in the progress of disease (Srinivasan, 2006). Fenugreek contains numerous dynamic phytochemicals, including vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin and saponins, which contribute to its antioxidant effects (Valette *et al*., 1984).

However, the research around the effects of fenugreek on the immunity of sows and piglets is still limited. The objective of this study was therefore to evaluate the effects of fenugreek on the blood profiles of sows and

piglets. The results of this study will add to the understanding of the mechanism by which fenugreek increases the immunity of piglets.

H_0: Fenugreek as a feed additive will not affect the blood profiles of piglets

H_1: Fenugreek as a feed additive will affect the blood profiles of piglets

3.2 Materials and Methods

3.2.1 Ethical clearance for animal use

This study complied with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008, and was completed with ethical clearance from the Stellenbosch University Care and Use Committee (SU ACUC), reference number: SU-ACUD15-00056.

3.2.2 Animals used in the study

The pigs used in this study were obtained from a commercial farm in the Western Cape Province of South Africa. The farm uses only PIC hybrid lines, which are produced by crosses between the Landrace, Large White and White Duroc breeds. They utilise a semen-only program and artificially inseminate with PIC line 337 boars to produce the terminal offspring and PIC line 2 boars for the maternal offspring.

The experimental outlay and diet fed to the pigs have been described in detail in Chapter 3. Briefly, the study used 120 sows and their 1480 piglets. All sows were moved to the dry sow house within the first five days of gestation, where they were housed in individual crates. The sows received a standard commercial dry sow diet during this time. The trial was done on two groups of 60 sows each, with varying farrowing statuses present within each group (gilt to eighth parity). Sows entered the trial at 85 days of gestation and on day 105 of gestation the sows were moved to the farrowing house. The sows were housed in an environmentally controlled house in individual farrowing crates. All sows received a commercial lactation diet until the piglets were weaned at 28 days old, which was the end of the trial. In the farrowing house, the piglets were fed a commercial piglet creep diet from day 10 until weaning.

For the blood analysis only the parity three and four sows and their litters were used. At weaning the whole litter was weighed and the piglet closest to the average weaning weight was used for blood sampling.

3.2.3 Feed characteristics

The feed used in the trial was a commercial feed produced by a commercial feed company. The feeding strategies and diet compositions are described in Chapter 3 (Tables Table **2.1**, Table **2.2**,Table **2.3Error! Reference source not found.**)

3.2.4 Treatments

Two commercial fenugreek products, Nutrifen® and Nutrifen Plus®, were used. Details of the two commercial products used can be found in Chapter 3 (2.2.4).

3.2.5 Blood collection and analysis

Blood was drawn from the sows and piglets in each batch on consecutive weeks due to the difference in the farrowing status of the sows and consequently the date of weaning of the piglets.

The blood sampling from the sows was done by an Animal Health Technician the day before the piglets were weaned. The sows were confined in their farrowing crates and a rope was placed in the sow's mouth behind the canine teeth in order to not apply pressure on the nasal cartilage, which is a sensitive area. As the animal moved backwards the rope was fastened, thereby restraining the sow. The blood samples were drawn from the jugular vein using Vacuette® 18 G x 1.5 inch (1.25 mm x 38 mm) needles with a plastic BD Vacutainer® holder and stored in 13 x 100 mm x 6.0 mL BD Vacutainer® Plus plastic whole blood tubes with Lavender BD Hemogard™ closure and with an additive of K_2EDTA (spray dried), 10.8mg (100/sp, 1000/ca). The samples were placed in a cooler box with an ice brick for transport.

Blood was drawn from the piglets just after weaning. The piglet was placed on its back and restrained. The blood was collected from the jugular vein using Improve® 21 G x 1.5 inch (0.8 x 38 mm) needles with a plastic Vacutainer® holder and stored in 13 x 100 mm x 6.0 mL BD Vacutainer® Plus plastic whole blood tubes with Lavender BD Hemogard™ closure and with an additive of K_2EDTA (spray dried), 10.8mg (100/sp, 1000/ca).

Immediately after collection, the blood was transported to the Stellenbosch University Physiology Department's laboratory and tested using a Cell-Dyn 3700 Hematology Analyzer.

After initial analysis the samples were centrifuged and the serum separated and stored at -20 ^{o}C until further analysis.

The thawed serum was analysed by the Hematology Department at the Tygerberg National Health Laboratory Service (NHLS). The instrument used was a Siemens Advia 1800, using the IGG_2 method, which is a polyethylene glycol-enhanced immunoturbidimetric method. The sample was suitably diluted and then reacted with a specific antiserum to form a precipitate that could be measured turbidimetrically at 340/694 nm. By constructing a calibration curve using the absorbance values for set concentrations of standard calibrators, the concentration of IgG was determined.

The whole blood was analysed for the following:

- White blood cells (WBC), $10^3/\mu\ell$,
- Red blood cells (RBC), $10^6/\mu\ell$,
- Lymphocytes, as a percentage of the WBC

The serum was analysed for the following:

- IgG, g/L,

3.3 Statistical Analysis

For analysing sow and litter blood profiles, ANOVA was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 2012), with the following dependant variables: white blood cell count, red blood cell count, lymphocyte and immunoglobulin G. The variables that were not normally distributed were log-transformed. The differences in the data was deemed significant when $P \leq 0.05$ and trends were noted when $0.05 > P > 0.10$. The variability in the data was expressed as the pooled standard error of the mean (SEM).

3.4 Results and discussion

3.4.1 White blood cell count (WBCC)

The activation of the immune system and inflammation may be detected by increases in specific markers in the blood, which include WBC (Vozarova *et al*., 2002). There was no effect of the fenugreek supplementation on the WBCC of the sows ($P = 0.841$) or the piglets ($P = 0.585$) at weaning. For all the treatments, the results were within the normal range (11 - 22 x 10^3/µL) for pigs (Aiello & Moses, 1998). This agrees with the study by Hossain *et al*. (2015), where no effect of feeding levels of 0.1 % and 0.2 % fenugreek seed extract was found on the WBCC but there was a significant effect on the RBCC of the piglets at weaning. The effect of the fenugreek supplementation on blood profiles of sows and piglets at weaning is presented in Table 3.1**Table 3.1**.

Table 3.1 Effect of fenugreek supplementation on blood profiles of sows and piglets at weaning

Item	Treatments			SEM	P Value
	Control	Nutrifen®	Nutrifen Plus®		
Sows					
White Blood Cell Count	14.85	14.38	14.17	0.320	0.841
Red Blood Cell Count	6.141	5.830	5.606	0.083	0.415
Lymphocytes	3.904	3.902	3.676	0.197	0.496
Immunoglobulin G	9.758	9.576	9.494	0.171	0.913
Piglets					
White Blood Cell Count	16.172	16.000	13.177	0.853	0.585
Red Blood Cell Count	6.486	6.071	5.379	0.160	0.081
Lymphocytes	8.533	6.077	5.161	0.744	0.262
Immunoglobulin G	2.936	2.818	2.557	0.147	0.583

3.4.2 Red blood cell count (RBCC)

The function of the red blood cells is to carry oxygen to tissues at pressures sufficient for rapid diffusion (Aiello & Moses, 1998; Klinken, 2002). Hossain *et al*. (2015) found that the RBCC of suckling piglets was improved by 0.2% fenugreek seed extract supplementation, possibly due to fenugreek's antioxidant activity increasing the stability of the red blood cell membranes through the arrangement of fatty acid complexes in the cell membranes. This would decrease the impact of free radicals (Erin *et al.,* 1984). In this study no effect of fenugreek supplementation on the RBCC of the sows (P = 0.415) and the piglets (P =0.081) at weaning was found. This can be because the supplementation levels were too low or the blood collection periods were not enough. The levels were within the normal range of 5–7 x 10^6/µL (Aiello & Moses, 1998).

3.4.3 Lymphocytes

Lymphocytes play an integral role in the immune system and are responsible for both cellular and humoral immunity (Muirhead & Alexander, 1998). The differences between the lymphocytes involved in the two functions cannot be seen morphologically but they have different actions in circulation and are produced by different tissues. The lymphocytes involved in cellular immunity originate from the thymus and then, under the influence of the thymic hormones, they differentiate further (Aiello & Moses, 1998). The T-Lymphocytes coordinate the acquired immune response by promoting intracellular neutralisation by macrophages, clonal expansion of cytotoxic T-lymphocytes and antibody production by B-lymphocytes (Fearon & Locksley, 1996). In the present study, treatment had no effect on the lymphocytes in either the sows (P = 0.496) or piglets (P = 0.262) at weaning. All the results were within the normal range (35%–75% of WBC: 3.8–16.5 x 10^3/µL) reported (Aiello & Moses, 1998).

3.4.4 Immunoglobulins

The principle antibody is immunoglobulin G (IgG), which is found in blood and extracellular fluid. This antibody controls the risk of transmitting blood-borne and other pathogens between body tissues (*Litman et al.,* 1993). In the present study fenugreek supplementation had no effect on the IgG levels of the sows (P = 0.913) or the piglets (P = 0.583). In contrast with these results, Ilsley *et al.* (2005) reported that piglets supplemented with quilaja (*Quillaja saponaria*) saponins for 20 days immediately after weaning had enhanced immune function, with IgA and IgG levels being increased in the treatment groups.

3.5 Conclusion

It was speculated that fenugreek supplementation should improve immune status but treatments did not have any significant effect on the blood parameters of the sows or piglets at weaning. This was not expected and further research is needed to establish the mechanism involved. In this study, blood collection was only done at weaning, and it would therefore make sense to do additional blood sampling during the late gestation period and early lactation period in sows in order to see whether the levels of these biomarkers increased or decreased over this period.

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Chapter 4

General Conclusion

The primary objective of this study was to evaluate the effects of fenugreek on sow reproductive performance and litter parameters. A number of performance parameters were used as response criteria to assess the effect of fenugreek supplementation during the last trimester of gestation until weaning at 28 days. This included the number of piglets born alive, the number of stillborn piglets, the number of mummified piglets, the litter birth weight (kg), the pre-weaning mortality (%) of the piglets, the piglets weaned per sow, the litter weaning weight (kg), the back fat thickness (mm) of the sows at weaning and the total feed intake during lactation (kg). There was no significant effect of the fenugreek supplementation on the response criteria measured. There was a significant effect on the piglets born alive but this was not a treatment effect. The only other significant influence found was on the back fat thickness of the sows at farrowing, but there was no significant effect on the back fat loss during lactation, which is considered more important for future reproductive performance.

When evaluating the effects of the fenugreek treatments for the same period as mentioned above on the blood profiles of the sows and their piglets no significant influence was found. The white blood cell count, red blood cell count, percentage of lymphocytes and immunoglobulin G levels did not differ significantly between the treatments for either the sows or their piglets.

In literature, there are several studies reporting the positive effects of fenugreek on milk production and immune system stimulation; however, the results of this study do not support this. It is therefore possible that the levels of fenugreek used were not adequate to influence the response criteria measured and further research is therefore needed to establish the correct inclusion levels for sows during gestation and lactation.